$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E.S \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E.P \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P$$

**Fig. 2** Simplified reaction scheme (E, PFK; S, ATP and F6P; P, ADP and F1,6BP). It can be shown that in the forward reaction assuming one substrate is saturating, the rate equation with respect to the other substrate has the following composite parameters (see for example ref. 19)) $k^f_{cat} = k_2k_3/(k_2+k_{-2}+k_3)$ and $K^f_m = K^f_s(k_3+k_{-2}+k_2k_3/k_{-1})/(k_2+k_{-2}+k_3)$, where $K^f_s = k_{-1}/k_1$ and corresponds to the dissociation constant for E.S. Of these terms, only $k_1$ and $k_{-1}$ (and hence $K^f_s$) are different for the two substrates F6P and ATP. Similarly, for the back reaction $k^b_{cat} = k_{-2}k_{-1}/(k_2+k_{-2}+k_{-1})$ and $K^b_m = K^b_s(k_{-1}+k_2+k_{-2}k_{-1}/k_3)/(k_2+k_{-2}+k_{-1})$, where $K^b_s = k_3/k_{-3}$. Constants $k_3$, $k_{-3}$ and $K^b_s$ are different for F1,6BP and ADP.

measurements. Nevertheless, by considering the changes in $k_{cat}$ and $K_m$ caused by the mutations, we can assign the changes to the different stages of the reaction, even if we cannot quantify the effect on the rate constants.

The Ser 127 mutant has greatly diminished $k_{cat}$ values in both directions of the reaction (Table 1). Much of this large change can be ascribed to the catalytic step ($k_2$ and $k_{-2}$ in Fig. 2), because the rate for the mutant enzyme is too low for rate-limiting product release ($k_3$, which might also contribute to $k_{cat}$). The much smaller decrease in $k_{cat}$ caused by the Arg→Ser 171 mutation is probably also due to changes in the catalytic rates $k_2$ and $k_{-2}$, because the changes in $K^f_m$, the Michaelis constants for the forward reaction, are small and similar for the two substrates.

Values of $K^f_m$ are changed only a little by either mutation and by about the same amount for the two substrates F6P and ATP. This is most simply ascribed to a change in $k_2$, with little change in the dissociation constants $K^f_s$ for the two substrates, because it is unlikely that a mutation would affect $K^f_s$ equally for both substrates. On the other hand, the $K^b_m$ values for the reverse reaction change by different amounts: $K^b_m$ (F1,6BP) is reduced by a factor of 45 in the Ser 127 mutant, whereas $K^b_m$ (ADP) is reduced by less than fourfold. This suggests that the removal of the Asp 127 carboxyl group in this mutant has stabilized the complex E.P in Fig. 2 and reduced the dissociation constant $K^b_s$ (F1,6BP).

The large changes in $k_{cat}$ when the carboxyl group of Asp 127 is removed are probably due to its acting as a general base in the forward reaction and as a general acid in the reverse reaction. Such a mechanism has been suggested, for example, for hexokinase[13,14] and staphylococcal nuclease[15]. General acid catalysis has also been demonstrated in the hydrolysis of salicyl phosphate[16]. We cannot exclude the possibility that the carboxyl group acts simply to orientate the accepting nucleophile correctly, but significant structural changes are unlikely, because the changes in $K_m$ are small. This aspartate is not (in the ADP complex) close to the $Mg^{2+}$ ion, which probably binds to Asp 103, so the effect of the mutation is unlikely to be due to changes in $Mg^{2+}$ binding. The change in $K^b_m$ (F1,6BP) shows that the Asp 127 side chain also destabilizes the E.F1,6BP.ADP product complex (E.P in Fig. 2), presumably by repulsion between the negatively charged carboxyl and the newly formed 1-phosphate on the sugar, thus aiding the release of products. It is not possible from these data to know whether in the wild-type enzyme the chemical step or product release is rate limiting in the forward reaction, but the presence of the Asp 127 side chain speeds up both steps.

Contrary to our expectations, Arg 171 does not appear to be of major importance in stabilizing the transition state. The evidence from non-enzymatic catalysis on the importance of such electrophilic catalysis is equivocal[17,18]. In many model systems, phosphoryl transfer seems to occur by a dissociative mechanism (forming a metaphosphate ion), but in an enzyme the distinction between associative and dissociative mechanisms is less clear, because the accepting nucleophile needs to be in place before the phosphoryl group leaves ATP (ref. 3), and the observed inversion of the phosphoryl group would seem to favour an associative pathway. Positively charged groups near the phosphoryl group would be expected to slow down a dissociative pathway, but speed up an associative pathway. The small catalytic effect we see here from Arg 171 is insufficient to make a distinction between these alternatives. Other positively charged residues in the active site near the γ-phosphoryl group, such as Arg 72, may prove important for this aspect of the catalytic mechanism. Further mutations will have to be constructed to investigate this.

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# Erratum

## Isolation of an excision product of T-cell receptor α-chain gene rearrangements

**Shinji Fujimoto & Hideo Yamagishi**

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Figure 1 was not printed, it appears here with its legend.

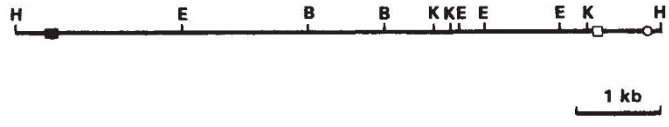


**Fig. 1** Restriction of a p113 spc-DNA insert containing the fragment rearranged for the genomic sequence. Restriction sites are indicated by vertical lines above the horizontal line. Restriction enzymes: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I. The location of a short interspersed repetitive B1 sequence (■) was determined by Southern blot analyses and the locations of $J_1$ (□) and the 'reciprocal joint' (○) were determined by sequencing the 0.85-kb *Kpn*I-*Hin*dIII fragment.